Influence of Smartphone Wi-Fi Signals on Adipose-Derived Stem Cells

Sang-Soon Lee, MD, PhD, Hyung-Rok Kim, MD,* Min-Sook Kim, MS,* Sanghoon Park, PhD,† Eul-Sik Yoon, MD, PhD,* Seung-Ha Park, MD, PhD,* and Deok-Woo Kim, MD, PhD**

Abstract: The use of smartphones is expanding rapidly around the world, thus raising the concern of possible harmful effects of radiofrequency generated by smartphones. We hypothesized that Wi-Fi signals from smartphones may have harmful influence on adipose-derived stem cells (ASCs). An in vitro study was performed to assess the influence of Wi-Fi signals from smartphones. The ASCs were incubated under a smartphone connected to a Wi-Fi network, which was uploading files at a speed of 4.8 Mbps for 10 hours a day, for a total of 5 days. We constructed 2 kinds of control cells, one grown in 37°C and the other grown in 39°C. After 5 days of Wi-Fi exposure from the smartphone, the cells underwent cell proliferation assay, apoptosis assay, and flow cytometry analysis. Three growth factors, vascular endothelial growth factor, hepatocyte growth factor, and transforming growth factor-β, were measured from ASC-conditioned media. Cell proliferation rate was higher in Wi-Fi–exposed cells and 39°C control cells compared with 37°C control cells. Apoptosis assay, flow cytometry analysis, and growth factor concentrations showed no remarkable differences among the 3 groups. We could not find any harmful effects of Wi-Fi electromagnetic signals from smartphones. The increased proliferation of ASCs under the smartphone, however, might be attributable to the thermal effect.

Key Words: Adipose-derived stem cell, electromagnetic, smartphone, Wi-Fi, growth factor, proliferation, apoptosis

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The use of mobile phones is expanding rapidly worldwide, and possible harmful effects of radiofrequency (RF) energy generated by mobile phones are of great concern to public health.[1]

Traditional mobile phones were used mainly for voice communication, and the close location of the RF-emitting phone to the head raised concerns about the relation between mobile phone use and brain tumor.[2,3] Recently, popularized smartphones are also used for voice communication, but people tend to spend more time compared with tradition mobile phones for the purpose of data communication. Smartphones make use of more than 2 frequency bands of RF, with 2.4-GHz Wi-Fi being the most commonly used signal for data transmission. Data transmissions through Wi-Fi signals are popular because of easy availability of free access points and unlimited data usage. Power output of 2.4-GHz Wi-Fi signals is restricted to 100 mW in many countries, which is less than those of other bands of RF signals supplied by mobile carriers (100–500 mW).[4] However, there has been concern that Wi-Fi signals might have harmful influence on human health. In addition, a recent study supported this idea of possible harmful effects of Wi-Fi signals. It showed that Wi-Fi signals from laptop computers had harmful effects on human sperm.[5]

The amount of RF exposure is inversely related to the square of the distance from the source.[4] If the distance from the RF-emitting device is the main factor responsible for the biologic effects without consideration of tissue vulnerabilities, skin and subcutaneous tissues will be the most affected tissues. Many studies have been conducted regarding the biologic effects and safety issues related to RF energy. The immune system, brain tissue, bacteria cells, and prenatal exposure have been extensively studied to determine biologic response to RF exposure.[1,6–10] However, only few studies have been undertaken on embryonic stem cells,[11–13] and no report has been done regarding the effect of RF on adipose-derived stem cells (ASCs).

The ASCs are multipotent cells involved in tissue repair and regeneration. These cells can undergo adipogenic, chondrogenic, osteogenic, and neurogenic differentiation.[14] In addition, ASCs produce various cytokines such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and transforming growth factor-β (TGF-β).[15,16] Moreover, there is a growing opinion that a large part of the beneficial effects of cell therapy are due to the secretion of cytokines.[15–18]

We used ASCs to investigate cellular responses to Wi-Fi signals for the following reasons. Superficial locations of the cells will receive more RF energy than other cells in the human body. The ASCs grow rapidly in vitro culture, and these rapidly growing cells will be more vulnerable to harmful external stimuli. Multipotent cells will display latent characteristics of differentiated cells in the body and can be studied further for the effect of RF on the differentiation potential of ASCs.

To assess the influence of Wi-Fi signals from a smartphone, we performed an in vitro study. The ASCs were incubated under a smartphone connected to a Wi-Fi network sending gigabytes of file data.

MATERIALS AND METHODS

Isolation of ASC

Human abdominal fat tissues were obtained from 5 patients who underwent abdominoplasty or breast reconstruction surgery. The study was approved by the institute of review board of Korea University Ansan Hospital. Informed consent was taken from all patients for use of their tissues.

From the *Department of Plastic and Reconstructive Surgery, Korea University Medical Center; and †Korea Electronics Technology Institute.
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Address correspondence and reprint requests to Dr. Deok-Woo Kim, Department of Plastic and Reconstructive Surgery, Korea University Ansan Hospital, 123 Jeokgeum-ro, Danwon-gu, Ansan-si, Gyeonggi-do 425-707, Republic of Korea; E-mail: deokwookim@gmail.com
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Subcutaneous fat was washed with phosphate buffered saline (PBS) and cut into small pieces of less than 1 mm using scissors. Adipose tissues were then treated with PBS containing 0.05% of collagenase type I (Sigma-Aldrich, St. Louis) under gentle agitation for 1 hour at 37°C.

The digested fat was centrifuged at 300 g for 10 minutes, and the ASC fraction was washed with PBS containing 1% of penicillin/streptomycin (Gibco, NY). The sample was centrifuged again at 300 g for 5 minutes. The supernatant was discarded, and cell pellet was resuspended in Dulbecco modified eagle's medium (DMEM; Hyclone, Logan, UT) containing 10% of fetal bovine serum (Hyclone, Logan, UT) and 1% of penicillin/streptomycin and then filtered through a 70-μm nylon mesh. The filtered cell fraction was incubated overnight, and adherent cells were collected. The cells were further cultured for passage 3 and used for the next step.

Incubation of ASCs and Wi-Fi Signal Exposure

The ASCs were plated on culture plates at a density of 1.5×10^3 cells/cm^2. The cells were maintained in DMEM containing 10% of fetal bovine serum and 1% of antibiotics at 37°C, in 5% of CO_2 environment. Twenty-four hours after cell plating, the cells were incubated under a smartphone (SHW-M110S; Samsung, Seoul, Korea) connected to a Wi-Fi local area network (IEEE 802.11 g, 2.4 GHz). The smartphone worked actively, uploading files at 600-KB/s speed for 10 hours a day, for a total of 5 days. Distance from the bottom of the smartphone to the adherent cells was 2 cm (Fig. 1). The mean power density of Wi-Fi signals was 26 μW/cm^2, and the calculated specific absorption rate (SAR) was 240 mW/kg. The SAR is defined as $\sigma E^2/\rho$ (W/kg), where E is the electric field strength, σ is the electric conductivity (S/m) for the frequency, and ρ is the sample density (kg/m^3).

After 5 days of exposure, the ASCs were harvested for proliferation, apoptosis, and flow cytometry analysis. The culture media were cryopreserved for growth factor analysis.

In the preliminary study, we found that the temperature of the culture media under the working smartphone rose approximately 2°C in 2 hours. Thus, we categorized 2 groups of control cells: cells incubated at 37°C were defined as normal temperature control, and cells incubated at 39°C were defined as high temperature control. Temperature changes of the culture media were serially recorded using a temperature probe inside the culture dish.

Cell Proliferation Assay

Cell proliferation was assessed using Cell Counting Kit 8 (CCK-8; Dojindo, Japan) and total DNA measurement. For CCK-8 assay, ASCs were incubated in 96 well culture plates. The culture medium was changed with 100 μl of serum-free DMEM before the assay. The cells in serum-free medium were added with 10 μl of CCK-8 solution and incubated for 2 hours at 37°C. Absorbance was measured at 450 nm using a microplate spectrophotometer (uQuant; BioTek, VT).

For the total DNA measurement, ASCs were homogenized in Trizol (Gibco, NY), mixed with 120 μl of chloroform by shaking vigorously, and then incubated for 15 minutes at room temperature. The sample was centrifuged at 3000 g for 15 minutes, and any remaining aqueous phase was removed. The remaining sample was added with 180 μl of 100% ethanol and then centrifuged. The precipitated pellet was washed with sodium citrate/ethanol solution and centrifuged again. The pellet was suspended with 1 mL of 70% ethanol, centrifuged, air dried, and resuspended with 20 μl of 8-mM sodium hydroxide. Absorbance was measured at 260 nm using a spectrophotometer (ND-1000; Nanodrop Tech, DE).

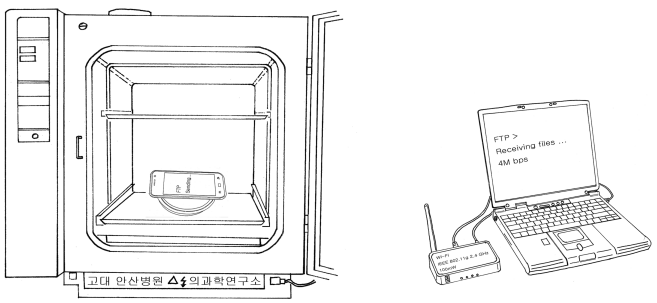

FIGURE 1. Study design for exposure of ASCs to Wi-Fi signals from a smartphone. The ASC culture plate was located under the smartphone, and the cells were incubated under the influence of a 2.4-GHz Wi-Fi electromagnetic field in a CO_2 incubator. The smartphone uploaded data files at a rate of 4.8 Mbps to a laptop connected to a wireless router.

Quantification of Apoptosis

Cell death was detected using Cell Death Detection ELISA plus kit (Roche, Mannheim, Germany). Briefly, the cells were incubated with 200 μl of lysis buffer for 30 minutes. After centrifugation at 200g for 10 minutes, 20 μl of the lysate was transferred into a strepavidin-coated 96-well microplate. Immunoreagent of 80 μl was added to each well and incubated for 2 hours. Unbound compounds were removed by washing with buffer supplied with the kit. 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) solution of 100 μl was added to each well, and the samples were incubated for 20 minutes. 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) stop solution of 100 μl was added to each well, and absorbance was measured at 450 nm.

Growth Factor Analysis

The ASCs were incubated in 60-mm culture plates with 3 mL of growth medium under the influence of Wi-Fi electromagnetic waves. The supernatant was centrifuged at 300 g for 5 minutes and then filtered. Concentration of the following cytokines were measured using sandwich ELISA kits (R&D Systems, Minneapolis, MN): HGF, TGF-β1, and VEGF.

An activation step was preceded for TGF-β1: 100 μl of the supernatant was mixed with 20 μl of 1 N of hydrochloric acid and neutralized with 20 μl of 1.2 N of sodium hydroxide/0.5 M of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Assay diluents RD1W was added to a 96-well microplate coated with specific monoclonal antibody, followed by the addition of 50 μl of the standard and supernatant samples to each well. After 2 hours of incubation, each well was washed with washing buffer. Peroxidase conjugated specific antibody was added to each well and incubated for 2 hours. After washing, substrate solution was added to each well and incubated for 30 minutes. Stop solution was added, and absorbance was measured at 450 nm.

Surface Antigen Analysis

We performed flow cytometry analysis for 2 mesenchymal stem cell markers, CD73 and CD105, and 2 negative markers, CD45 and CD31.

Cells were collected and centrifuged at 300 g for 5 minutes, and the pellets were resuspended in PBS. Aliquots containing 1×10^5 cells were incubated with fluorescence-conjugated anti-CD105, anti-CD73, anti-CD45, and anti-CD31 antibodies (BD Bioscience, San Jose) for 1 hour at room temperature. The cells were precipitated by centrifugation at 300 g for 5 minutes and washed in PBS. The 4 surface antigens were analyzed using the flow cytometry system (FACSCalibur; BD Bioscience, San Jose) with CellQuest Pro software.

Statistical Analysis

Statistical analysis was performed with SPSS 12.0 software (SPSS Inc, Chicago, IL). Two nonparametric tests were used for analysis. The Friedman test was used to determine the differences among the 3 groups, and the Wilcoxon signed-rank test was used for post hoc comparison. A $P < 0.05$ was considered statistically significant.

RESULTS

Temperature Change of Culture Medium

The temperature setting of the CO_2 incubator was 37°C. A temperature probe was soaked in culture medium of the 60-mm culture plate. The temperature of the culture medium rose after the smartphone started to upload data and reached a steady state in 2 hours. The temperature change of the medium under the smartphone was 2.0°C. The temperature changes of the medium on the smartphone and beside the smartphone were 1.9°C and 1.2°C, respectively (Fig. 2).

Cell Proliferation Assay

Wi-Fi–exposed cells showed a higher proliferation rate than 37°C control cells. Both CCK-8 assay and total DNA amount results were statistically significant ($P < 0.05$). However, cells incubated in the 39°C environment also showed increased cell proliferation in the 2 tests compared with 37°C control cells ($P < 0.05$). There were no significant differences between Wi-Fi–exposed cells and 39°C control cells (Figs. 3, 4).

Quantification of Apoptosis

Apoptosis assay showed a decreasing tendency of cell death in both Wi-Fi–exposed cells and 39°C control cells compared with 37°C control cells. However, the P value was not statistically significant ($P > 0.05$) (Fig. 5).

Growth Factor Analysis

Wi-Fi exposure had no influence on the growth factor secretion of ASCs. The TGF-β1 concentrations in 37°C control cell medium, 39°C control cell medium, and Wi-Fi–exposed cell medium were 1253 (142), 1219 (130), and 1198 (64) pg/mL, respectively. The VEGF concentrations were 247 (60), 265 (80), and 255 (92) pg/mL in each group. The HGF concentrations were 62 (7), 64 (8), and 61 (5) pg/mL in each group. There were no significant differences in growth factor concentrations among the 3 groups (Fig. 6).

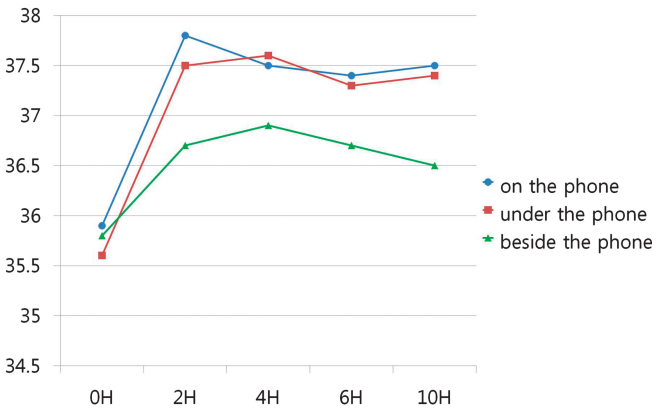

FIGURE 2. Temperature changes of culture media around the smartphone working in a Wi-Fi network. The temperature rose as the phone started working; the peak temperatures were 1.2°C to 2.0°C, which were higher than baseline.

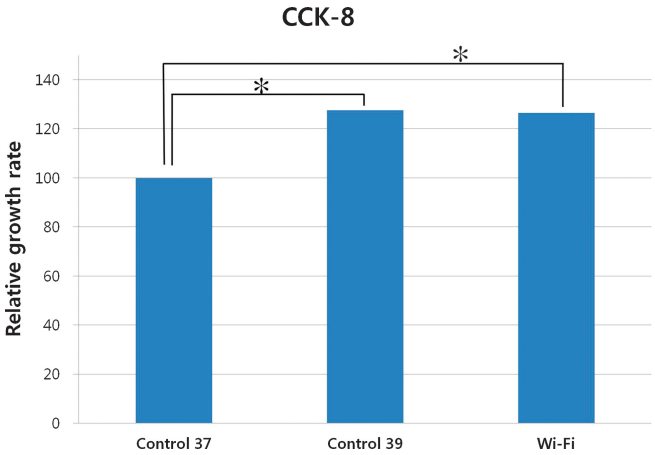

FIGURE 3. Growth rates of ASCs measured by CCK-8 level in 3 different conditions. The relative growth rate of Wi-Fi–exposed cells was significantly higher than control cells incubated in the 37°C environment. However, control cells incubated at 39°C showed the same results. *Significant difference for $P < 0.05$.

Surface Antigen Analysis

Flow cytometry analysis showed no differences in the frequencies of CD105, CD73, CD45, and CD31 positive cells among the 3 groups. Two stem cell markers (CD105 and CD73) were consistently stained positive, and 2 negative markers (CD45 and CD31) were almost undetectable in all cell groups (Fig. 7; Table 1).

DISCUSSION

Wi-Fi communication is based on the pulses of RF signals with no RF between bursts.[19] The fraction of time for RF signal transmission (duty cycle) is usually low; thus, time-averaged power output becomes far lower than peak output power (100 mW in many devices). When Wi-Fi devices are not transferring data, there are only beacon signals that correspond to a duty cycle of 0.01%. The duty cycle increases when a user transmits data through Wi-Fi, and the RF power output of Wi-Fi client is 10 times higher during uploading than downloading.[4] In this study, we continuously

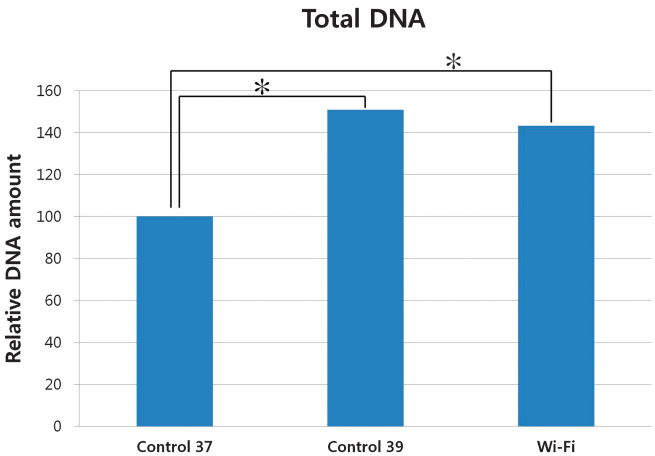

FIGURE 4. The total DNA amount of cultured cells, which reflect that cell replication was significantly higher in both Wi-Fi–exposed cells and control cells incubated at 39°C. *Significant difference for $P < 0.05$.

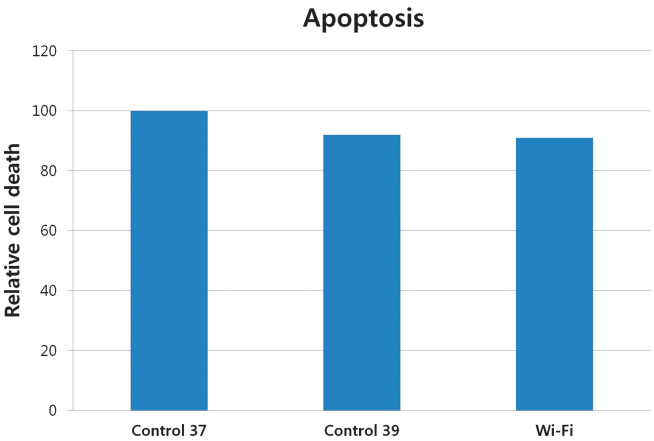

FIGURE 5. Apoptotic cell death measured by histone-associated DNA fragmentation was relatively low in both Wi-Fi–exposed cells and control cells incubated at 39°C. However, there was no statistical significance ($P > 0.05$).

uploaded file data from the smartphone at a rate of 600 KB/s, which means that RF exposure to cells (SAR of 125 mW/kg) would be much more than that of ordinary use in downloading applications or web surfing.

The RF produces heat when absorbed in tissues or water. The RF signals between 2.4 and 2.5 GHz are also used in microwave ovens of which the power is 10,000 times greater than that of Wi-Fi. In our study, the temperature of the medium rose approximately 2°C when the smartphone uploaded data through Wi-Fi signals. Absorption of Wi-Fi signals by the culture medium would contribute to the elevated temperature of the medium. However, direct transfer of thermal energy from the heated smartphone might be another cause of thermal elevation of the medium.

Cellular response to RF exposure includes DNA damage, generation of heat shock proteins, apoptosis, and gene/protein expression changes.[1] Cytogenetic DNA damage is the most concerned problem of RF energy. However, there is a lack of evidence for possible DNA damage after exposure to RF energy below the basic restrictions for the human body (SAR of 2 W/kg). Many experimental studies have reported that exposure of mammalian cells to RF did not result in increased cytogenetic damage.[20–23] Other studies have reported possible cytogenetic changes, but the powers of RF were much larger than the restricted values for body exposure.[24,25]

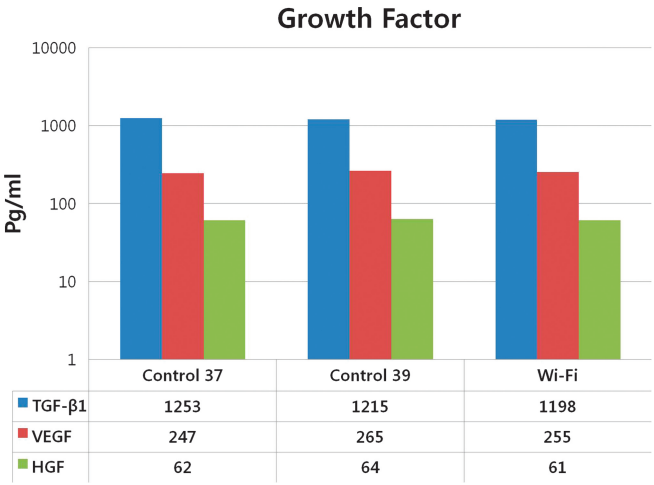

FIGURE 6. Growth factors released by ASCs incubated under 3 different conditions. There were no significant differences in growth factor concentrations among the 3 groups ($P > 0.05$).

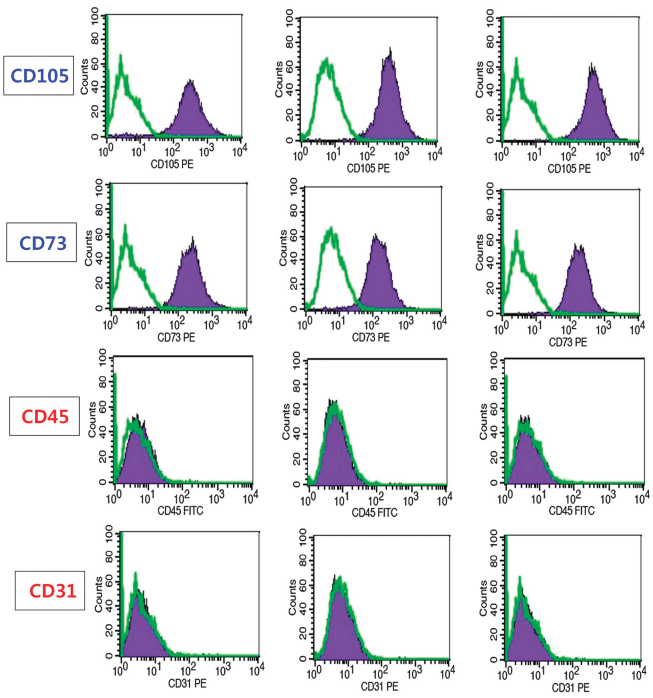

FIGURE 7. Flow cytometry analysis of ASCs incubated under 3 different conditions. The surface antigen expressions of the 3 cell groups showed similar patterns.

Heat shock proteins are a group of proteins that are expressed in response to stress conditions such as heat, cold, exposure to toxic chemicals, and other environmental insults.[1] These proteins are known to contribute to heat tolerance and perform essential functions for cell survival under stress conditions.[10] These proteins have been proposed as possible stress markers of RF exposure, and there have been a large number of studies on the relation between RF exposure and heat shock protein release. However, most research did not support the role of nonthermal RF radiation in the generation of cellular stress proteins.[10,26] Strict temperature control is essential for studying the nonthermal effects of RF on heat shock protein release. However, some studies with positive results seemed to have had difficulties in preventing temperature changes.[27,28] Our study was planned to make an experimental setup similar to the normal conditions of Wi-Fi signal exposure during smartphone use, and the steady control of temperature was not intended. The warming of cutaneous tissue nearby the phone itself was regarded as an influence of smartphone and Wi-Fi signals.

Apoptosis of RF-exposed cells was investigated in many studies, and most of them revealed that the apoptosis was not increased in the RF-exposed cell culture model. However, recently published studies on human sperm, which was incubated under a laptop computer connected with a 2.4-GHz Wi-Fi network, showed increased apoptosis measured by the terminal deoxynucleotidyl transferase dUTP nick end labeling assay. The sperms are heat sensitive; thus, the experimental results could be influenced by the heat from the laptop. Our results showed that apoptosis was not increased, but the growth rate was increased in both Wi-Fi–exposed cells and 39°C control cells. This enhanced cell proliferation can be attributable to the thermal effect from the smartphone. Hyperthermia in the physiologic range can enhance cell proliferation and differentiation depending on the cell type.[29] However, proliferation of ASCs has not been shown to be enhanced by hyperthermia

TABLE 1. CD Antigen Expression Rates of ASCs in Different Conditions

Surface Antigen	Control 37, %	Control 39, %	Wi-Fi, %
CD105	98.2 (1.3)	98.4 (2.7)	96.9 (3.5)
CD73	86.7 (9.9)	87.5 (5.5)	89.4 (4.8)
CD45	0.2 (0.1)	0.2 (0.1)	0.3 (0.5)
CD31	0.2 (0.1)	0.1 (0.1)	0.1 (0.1)

Data are presented as mean (SD). There was no statistical difference ($P > 0.05$).

in the physiologic range in other studies; thus, it will serve as a clue for studying the influence of hyperthermia on the proliferation and differentiation of ASCs.

Many studies have attempted to identify gene expression changes of various cells after RF exposure. Most studies were performed with the microarray technique and reported some changes in the messenger ribonucleic acid level.[10,26,30,31] However, microarray methods have high false-positive rates, and changes in the messenger ribonucleic acid level may not be expressed in the protein level. We tried to observe gene expression changes in the protein level. Concentrations of 3 outstanding cytokines were measured, and the expression levels of 4 CD markers were analyzed. The ASCs produce a significant level of angiogenic cytokines, which is the most important ASC characteristic that differentiates from fibroblasts.[16] The VEGF and HGF are angiogenic cytokines, and the levels of secretion were not changed by Wi-Fi exposure form the smartphone. The TGF-β1 plays an important role in fibroplasia and immune modulation and shows different activities on different types of cells or cells at different developmental stages. The TGF-β1 concentration of the ASC culture medium was approximately 1200 pg/mL and was not changed by experimental conditions. Some reports have postulated that specific therapeutic actions of ASCs such as collagen synthesis and whitening are mediated by TGF-β1.[32,33] However, the concentration of TGF-β1 (1200 pg/mL) in the ASC conditioned media was much lower than the human serum level (40 ng/mL).[34]

Freshly isolated plastic adherent cells from adipose tissue undergo remarkable changes in CD marker expression. The CD31, CD34, and CD45 expression rates rapidly fall as culture time progresses, and CD73, CD90, and CD105 expression levels dramatically increase.[35] After 3 passages of culture, flow cytometry analysis of ASCs exhibits a CD31-, CD34-, CD45-, CD73+, CD90+, and CD105+ pattern. In our study, Wi-Fi signals from the smartphone did not influence the surface marker expression pattern of ASCs.

We did not induce adipogenic, osteogenic, and chodrogenic differentiation of ASCs under Wi-Fi exposure, which remains to be examined in a further study.

CONCLUSIONS

We could not find any harmful effects of emitting Wi-Fi signals from a smartphone on human ASCs. The enhanced proliferation of ASCs under the smartphone, however, might be attributable to the thermal effect.

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